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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 14 FEBRUARY 1956 No. 12

A NUTRITION OFFICER'S EXPERIENCES VISITING THE SOUTHWEST PACIFIC ISLANDS

It was in answer to a request to investigate the nutritional status of Fijians and Indians in Fiji, and Samoans in Western Samoa, that Dr. Lucy Wills (on a "holiday" in New Zealand) and I set forth together in January 1950 for Fiji, with only eight weeks at our disposal, during which I went to Samoa as well. The terms of reference for the Fiji study (published in the Medical Department's Annual Report for 1950, *Government Press, Suva (1952)*) were centered around anemia, possible protein and vitamin deficiencies, and any nutritional clues that could be gathered to account for increased prevalence of dental caries. Initial fears that in such a brief survey one could readily be deceived by superficial acquaintance with the problems have now been succeeded by the mass of questions that need solution. Anyone starting on what appears to be something of limited scope soon finds it branching until it is a mass of hyphae each terminating in an interrogation mark.

Since time is short, a vacation study planned for islands 2000 miles away must be prepared well in advance—apparatus, chemicals, and as far as possible ready-made solutions (such as will remain stable) must be sent by boat, often two months in advance. Obtaining sufficient data for statistical analysis in a short time involves using field methods, especially so when much of the time for visiting a village is spent on courtesies—being received (with kava) by the Chief or his deputy, listening to and answering speeches of welcome, with translations of both the languages used, without which courtesies one would get nowhere.

In doing a survey on hemoglobin values and at the same time finding whether serum protein values could throw any light on possible subnormal protein intake, the copper sulfate method (R. A. Phillips *et al., U. S. Navy Bumed. News Letter, Vol. 1, p. 1 (1943)*) proved very useful. As it was necessary to avoid passing on infections the stilettes were sterilized in the flame from an alcohol burner. The whole village watched with great interest—they had never before seen blood withdrawn into a clear glass pipette. The adolescents paid respect by courteously kneeling, though it would have been easier on our own knees if they had remained standing: By the laws of hydrodynamics, blood flows in sufficient quantity to provide for an unhemolyzed sample of serum from a finger prick only if the hand is held down. A kneeling donor means also a lower-kneeling recipient—and the floors are made of pebbles!

Before embarking on a tour of the villages, however, it was important to gather impressions of what type and amount of clinical material of nutritional import turned up in the hospitals, particularly in the children's, maternity, and medical wards. Nothing seemed to answer to the description of kwashiorkor, and yet, a few weeks later, a case was accidently observed by Sir Phillip Manson-Bahr, who was at the time revisiting Fiji. Subsequently P. E. C. Manson-Bahr described 16 cases of kwashiorkor from Fiji (*Tr. Roy. Soc. Trop. Med. Hyg.* **44,** *555 (1951)*; *Documenta de Medicina Geographica et Tropica* **4,** *97 (1952)*).

The Fijians lead a communal life in villages, living on dalo (taro, *i.e., Colocasia*), yam (*Dioscorea*), tapioca (*Manihot*), breadfruit (*Artocarpus communis*), banana and coconut with occasionally some fish or land crabs. There are virtually no cows,

few chickens are kept, and no maize is eaten. Nevertheless, the Fijians are in the main a well-nourished race, in contrast with the Indians, in whom cases of nutritional deficiencies turn up in the hospital wards from time to time. The weaning period is nowadays a critical one for the Fijian infant. It was obvious, from the Health Sister's records, vital statistics, and the appearance of the infants in the postweaning period, that the Fijian infant was at a disadvantage compared with the Indian infant. Manson-Bahr states that in what the Fijians call the "older days" the age for completion of weaning was by custom fixed at two years; nowadays the age is one year. "With the breaking down of Fijian custom, the children are weaned earlier, and as no substitute such as cow's milk is available, a sudden deprivation of protein occurs, the infant is left at the end of the queue at the communal eating mat and has no chance of obtaining any of the choice protein foods which are consumed by the elder Fijians. The shortening of the suckling period partly owing to breakdown of custom and partly as a result of European teaching can thus result in the appearance of kwashiorkor.

"The Indians in Fiji among whom kwashiorkor is not found live in families which are usually large. The annual birth of a child is commonplace so that early weaning of the infant occurs, but there is an important difference, viz. cow's milk is available for the infant."

Several cases of kwashiorkor turned up following a destructive hurricane, giving the impression that shortage of total calories might also play a part in producing Fijian kwashiorkor.

It could well be that in our survey, which had to be brief, any case of illness in a Fijian child might have been kept out of sight. One medical practitioner there overcame the tendency of these people to hide the abnormal by keeping a card-index (with a card in readiness for the unborn child!), and then on visiting a village he was able to ask the Chief why the missing one had not answered the roll-call.

As to hemoglobin values, three groups showed a moderate degree of anemia: Fijian and Samoan children of the 6 to 9 age period, Indian women of the child-bearing age and Indian infants aged 1 to 2 years.

It was our impression that the high rate of skin sepsis due to infected mosquito bites in the 6 to 9 year old Fijian and Samoan children was playing havoc with the activity of the bone marrow. By contrast, the Indian child, though born with a lower hemoglobin value, soon gained ground and then surpassed the Fijian and Samoan child in hemoglobin status. We were inclined to ascribe the difference to the better intake of animal protein through the milk given to the Indian child.

A statistically significant difference in hemoglobin levels was found between Indian pregnant women and those of the Fijian and Samoan races—10 per cent of the former had values of under 60 per cent (8.7 g.), 25 per cent had 70 per cent (10.15 g.) while only 13 per cent attained the 90 to 100 per cent (13 to 14.5 g.) level, whereas the Fijian and Samoan women followed during pregnancy a pattern of hemoglobin levels similar to those found in the 1943 British hemoglobin survey (*Med. Res. Council (London) Special Report Series No. 252 (1945)*). Nonpregnant Indian women in the child-bearing period also had significantly lower hemoglobins than those in the corresponding series of Fijian women. The main factor producing the difference is the earlier age of marriage of Indian women and the short intervals between pregnancies. The anemia of the Indian infants was clearly due to shortage of iron in the mother, who was then unable to confer a normal prenatal store on the infant.

Though a number of cases of macrocytic anemia among Indians, chiefly in Indian

women, had been admitted to the hospitals, no cases were encountered during the survey in the villages or towns. The number of cases that had been recorded fell far short of the prevalence of the disease in India, as elucidated two decades previously by L. Wills (*Brit. Med. J.* **1,** *1059 (1931)*). It is true that the incidence of this disease undergoes a seasonal variation, being less in the season when the survey was made.

A further analysis of the figures for hemoglobin among the Fijians, comparing inland and coastal zone districts, showed that in every age group the mean values for the inland villages were lower than for the coastal zone, bespeaking, so we thought, the influence of better protein supplies on the general health through juxtaposition to the sea's fishery resources.

As to any factor of nutritional significance which might throw light on the recent tendency for Fijian teeth to decay, nothing definite emerged. The four top incisors of the primary dentition were often structurally defective enough to lead to the early breakdown of the teeth, which then decayed down to the level of the gums by the second or third year. This seemed to point to some hypoplastic condition arising *in utero*. The secondary dentition then appeared to be well formed.

In the maternity ward of the Colonial War Memorial Hospital at Suva, Fiji, the average birth weight of Indian infants, 5½ pounds, was in marked contrast to the 8 pound average birth weight of Fijian infants. To what extent the small size of the Indian infant, and indeed the small frame of the adult Indian, is of hereditary or nutritional origin has yet to be determined. In "Growth and Development of Mutton Qualities in the Sheep," J. Hammond has shown in sheep that young mothers which are themselves still growing usually produce smaller young at birth than do fully adult mothers. Furthermore, he has evidence from crossing cattle of different sizes that the larger dam, having a larger supply of nutrients, produces a heavier offspring than the smaller dam. In a personal communication, he expressed the opinion that the plane of nutrition and the age of the mother would have an undoubted influence on the size of the infant. It remains to be seen whether several generations of Indian mothers, habitually on a good diet and marrying when adolescence is completed, will eventually produce an increase in the average size of the Indian infant.

There is a well marked difference in the incidence of eclampsia in the two races in Fiji. This disorder of pregnancy is almost unknown in Fijians, Samoans, Solomon Islanders, or New Guinea natives (M. E. Bell and L. Wills, *J. Obstet. Gynec. Brit. Empire, in press*), whereas in the Indians living on the same island as the Fijians there is an incidence comparable with that experienced in most European races. The reason for this difference has not yet been elucidated. S. Holmes and E. Langley have been collecting data on the dietary habits of the two races in Fiji which will help to determine whether there are grounds for the thesis put forward by E. H. Hipsley (*Brit. Med. J.* **2,** *420 (1953)*) that something associated with the presence of a large amount of dietary roughage has a protective action in the above races, but details of the Indian dietary in this respect are not yet known.

As goiter had been endemic in New Zealand (though brought under control virtually within a decade by the voluntary use of iodized salt), it was natural to compare clinically the thyroids in Fiji with those in New Zealand. It had been stated at the outset that there was no goiter in Fijians. However, during a visit to a village that was inaccessible except on horseback, a woman with an obvious large adenoma of the thyroid was among the group examined. In reply to questions put to the Health Sister and to an Indian Medical Practitioner in that area, the answers were convincing, that goiter was fairly common among the

Indian women and not unknown among the Fijian women. A study of Indians and Fijians living in the same districts, both near the sea, showed that there was a marked racial difference in incidence. Thyroid hypertrophy was very prevalent in an Indian girls' school near Suva. It is easier to find goiter than to suggest an effective prophylaxis in an area such as Fiji, where there is not a common article of diet to which an iodine-containing compound can be added. The cause of the racial difference is not known, but should be sought either among possible goitrogens in the foods used exclusively by the Indians, or in comparison of iodine intakes or excretions as between the two races.

Only a few of the many questions raised by our survey have been related in the present outline. Out of such studies may come important slants on the errors of our own way of living. Obviously, the Island peoples have something to teach us about immunity to disorders of pregnancy and to dental caries, even though we can help them with some of our information about infant feeding. Grateful as I am to our rat colony for the tales it tells, "the proper study of mankind is man."

MURIEL E. BELL
Department of Nutrition Research
Medical School
Dunedin
New Zealand

CALCIUM INTAKE AND RICKETS

The possible relationship between a low level of calcium intake and the development of rickets in children has been reviewed by A. R. P. Walker (*Am. J. Clin. Nutrition* **3,** *114 (1955)*). He points out that a high intake of the element is often recommended on the grounds that a low intake causes or predisposes to the development of rickets. It is clear that in the rat, at least, a poor calcium:phosphorus ratio in the diet does produce rickets in the absence of vitamin D. Since typical rickets is not produced in the absence of vitamin D if the ratio is satisfactory, it appears that the unfavorable calcium:phosphorus ratio may actually increase the vitamin D requirement enormously.

It may be pertinent to note that the studies on rats have practically all been done with diets very high in calcium and low in phosphorus, a kind of diet probably never encountered in human nutrition. The other kind of unfavorable ratio, high in phosphorus and low in calcium, apparently does produce rickets in the rat (A. T. Shohl and S. B. Wolbach, *J. Nutrition* **11,** *275 (1936)*), but this type of experimental rickets is apparently less satisfactory for experimental work. Thus it would appear that although there is abundant evidence that vitamin D affects calcium absorption, even in the rat there is little evidence as to the quantitative relationships between vitamin D and the calcium requirement.

Whereas the nutritionists have been much impressed by the possible role of calcium intake on rickets, Walker remarks, "it is noteworthy that standard textbooks on pediatrics, in the main, do not appear to regard level of calcium intake of much significance," and some "authorities reject the implication of calcium deficiency in the etiology of the disease." Walker then considers the data available from various parts of the world and the problems associated with the diagnosis of rickets.

Four methods of diagnosis are considered. These are (1) clinical assessment, (2) radiologic assessment, (3) histopathologic examination of bone samples, and (4) the chemical analysis of bone. No mention of the use of an alkaline phosphatase test is made. Histopathologic and chemical analysis of bone can obviously only be used upon postmortem material. Chemical analysis is of doubtful value since marked changes in

mineralization are only found in severe rickets, which can be readily diagnosed by clinical methods (A. R. P. Walker and U. B. Arvidsson, *Metabolism* **3,** *385 (1954)*). Microscopic examination is much more sensitive, but there are differences in the grading of the material by various workers. Walker states that should the severe criteria used by R. H. Follis, E. A. Park, and D. Jackson (*Bull. Johns Hopkins Hosp.* **91,** *480 (1952)*) be applied in many less privileged areas of the world, there would be very few children graded as free of rickets. Severe rickets is easily detected by clinical and radiologic examination, but this is not true of mild rickets. The author stresses the poor agreement between clinical and radiologic assessments as indicated by the British Pediatric Survey ("The Incidence of Rickets in War-Time," *Reports on Public Health and Medical Subjects, No. 92, Ministry of Health, London (1944)*). Poor mineralization can arise from several causes and, as J. B. Youmans has indicated (*J. Am. Med. Assn.* **143,** *1252 (1950)*), osteoporosis has often been uncritically interpreted as being the result of an inadequate calcium intake.

A consideration of reports from various areas of the world shows that in many tropical areas the calcium intake is habitually low but the incidence of rickets is also very low. However, in crowded urban areas of Africa and India rickets is common and occasionally severe. In Johannesburg rickets is reported frequently in breast-fed babies whose calcium intake is presumed to be normal. The incidence is low after 2 years of age when the child begins to fend for himself, although at that time the calcium intake (compared to recommended allowances) is very low. Osteomalacia in pregnant Bantu women has not yet been reported in Central or Southern Africa. The disease in India and China is apparently related to exposure to sunlight but not to the calcium intake. In northern countries where the available radiation is much less, rickets may be common. Again, in studies in Glasgow and Scandinavia quoted by Walker, there is no evidence that calcium intake is a determining factor in the susceptibility to the disease.

Walker concludes that although there is no available evidence which indicates that calcium intake is an etiologic factor in the development of rickets, the possibility that it does exert an influence cannot be completely excluded. In any event "it would seem wholly unwarranted to list prophylaxis against rickets as a reason for insisting on high calcium intakes" as presently recommended. The time is opportune for a reexamination of the whole position relating to calcium requirements and calcium deficiency.

HUMAN PANTOTHENIC ACID DEFICIENCY

The clinical and metabolic results of the administration of a pantothenic acid antagonist (omega-methyl pantothenic acid) to human volunteers on a pantothenic acid-deficient diet by W. B. Bean and R. E. Hodges have been discussed in *Nutrition Reviews* **13,** *36 (1955)*. Further details of the study have now been presented by Bean, Hodges, and K. Daum (*J. Clin. Invest.* **34,** *1073 (1955)*), including in a second paper, reported by G. H. M. Thornton, Bean, and Hodges (*Ibid.* **34,** *1085 (1955)*), a study of gastric secretion and motility. The present report concerns only 3 of the 4 "normal" subjects of the original report, presumably the 3 on whom the largest number of metabolic studies were completed. The clinical and metabolic observations in the first report are confirmed in the present articles and more of the data is given.

The clinical symptoms and signs, especially neurologic ones, were particularly noteworthy. Easy fatigability and som-

nolence progressed to a quarrelsome and sometimes irascible state. Paresthesias of the hands and feet became progressively worse and abnormality of gait occurred. Almost continual upper respiratory infections were noted, particularly acute pharyngitis.

The biochemical changes reported include impaired ability to acetylate para-aminobenzoic acid (PABA), reduction in blood cholesterol and cholesterol esters, and a decreased eosinopenic response to ACTH. The latter was considered to result from decreased adrenal cortical function. In addition, an increased sensitivity to insulin and a reduction of urinary 17-ketosteroid excretion were observed.

The data concerning the acetylation of PABA (Bean *et al.*, *loc. cit.*) do not show a consistent or marked reduction in acetylation during the deficiency state. After twenty-five days of deficiency, when the patients were at the height of their symptoms, massive doses of pantothenic acid were administered (4 g. per day). It was during this time that the acetylation of PABA reached its lowest levels in 2 of the 3 patients. Hence the studies of the acetylation of PABA, although suggestive, do not appear to be conclusive.

An increased sensitivity to insulin was observed in all patients, although it is not clear from the results on the one patient for whom data is presented that severe hypoglycemia did occur during deficiency as compared to the periods before deficiency and after recovery from deficiency.

The eosinopenic response to ACTH administration apparently disappeared during the period of pantothenic acid deficiency. This was accompanied by a progressive rise in blood eosinophil counts during this period. Again, this rise appeared to continue after the administration of pantothenic acid while the metabolic antagonist was continued. A similar paradox is noted with respect to cholesterol and cholesterol esters. It is no surprise that there was a progressive reduction in these substances in the blood during the period of feeding the special diet, as it was very low in both fat and cholesterol. However, a further drop in cholesterol esters not reflected in the total cholesterol occurred during the period of high dosage of pantothenate when the antagonist was continued.

The urinary 17-ketosteroid excretion decreased somewhat during the period of pantothenate deficiency and antagonist administration, but decreased to its lowest level during the period that pantothenate was administered in large doses. Likewise, clinically the subjects did not improve when the massive doses of pantothenate were given. Symptoms suggestive of adrenal cortical deficiency occurred at this time, so that the patients were given cortisone and then a nutritious diet, with marked clinical and metabolic recovery thereon.

In the second study careful investigation was made of gastric secretion and motility during the experimental periods. Marked decrease in gastric secretion as well as a reduction in the acid content and some decrease in the pepsin content of the gastric juice were observed in all 3 patients and were most marked in 2 of them. Following administration of the massive doses of pantothenate, there was some return toward normal gastric function in most instances. However, the most dramatic return to normal occurred when the metabolic antagonist was omitted and a well-balanced normal diet supplemented with vitamins was administered.

The authors emphasize the small number of subjects and the considerable variation observed. It was also noted and emphasized in the previous review that a completely favorable response was not obtained when pantothenate in large amounts was given, but only following discontinuance of the antagonist and resumption of a general diet supplemented with vitamins and cortisone. In fact, the patients appeared to be clinically and metabolically at their

worst during the period of massive pantothenate administration with continuation of the antagonist. This raises the question whether some of the untoward effects might have been due to some other "toxic" effect of the metabolic antagonist. The metabolic studies given in the complete report support this explanation in that the most marked abnormalities occurred after pantothenate was again administered in large amounts even though the antagonist was continued.

There is no doubt that these volunteers became very ill during the study period of pantothenate deficiency and antagonist administration. It may well be that it takes some time to repair this state of deficiency, and that it continues in spite of the administration of massive quantities of pantothenate. Whatever the explanation, no doubt exists that a severe illness occurred associated with a purified diet, omission of pantothenate, addition of the antagonist, and supplementation with thiamine, riboflavin, pyridoxine, niacin, cyanocobalamin, ascorbic acid, vitamins A and D, and salts. The conclusion that pure, uncomplicated pantothenate deficiency alone accounts for the change is not entirely justified.

THE EFFECT OF SITOSTEROLS ON THE SERUM CHOLESTEROL OF MAN

Feeding chicks a diet rich in cholesterol regularly results in hypercholesterolemia and, if continued, arterial atheromatous lesions ensue. Investigators in this field have sought agents which would prevent the complications of cholesterol feeding in chicks with the hope that such agents might be useful in the control of atherosclerosis in man (*Nutrition Reviews* **12,** *54* (*1954*)). Among the compounds which have been investigated have been certain analogues of cholesterol such as the phytosterols from soybeans and the animal sterol, dihydrocholesterol. It is known that the gastrointestinal absorption of these compounds is very small and it was hoped that the absorption of cholesterol might thus be competitively blocked. A protective action of soybean sterols in cholesterol-fed chicks has been reported by D. W. Peterson (*Proc. Soc. Exp. Biol. Med.* **78,** *143* (*1951*)) and a similar action was demonstrated in rabbits by O. J. Pollak (*Circulation* **7,** *696* (*1953*)).

The application of these experimental studies to man is a subject of great current interest. O. J. Pollak (*Circulation* **7,** *702* (*1953*)) administered a mixture of soybean sterols, composed largely of several types of sitosterol, to 26 patients. The daily dose of sterol amounted to 5 to 10 g. and the period of study varied from eight days to eight months. No attempt was made to control the diet of these subjects and the changes in body weight are not recorded. Although unpleasant to take, the soybean preparation appeared to be without toxic effect. From the small amount of data presented, the total serum cholesterol appears to have been lowered in individuals with initially elevated values.

One of the major components of the soybean sterol mixture is beta sitosterol, and its effect on the serum cholesterol has been studied by M. M. Best, C. H. Duncan, E. J. Van Loon, and J. D. Wathen (*Circulation* **10,** *201* (*1954*)). Beta sitosterol differs from cholesterol only in having an ethyl group attached to carbon atom 24 of the side chain. A control study of the effect of sitosterol was attempted in 9 subjects. Seven of these 9 individuals had elevated cholesterol levels of varied etiology and 2 were normocholesterolemic. The period of observation extended for thirteen to twenty-nine weeks, during which time periods of sitosterol administration (5 to 6 g. before each meal) were alternated with control periods during which a placebo of similar appearance was given. The mean total serum cholesterol fell between 6.7 and 20

per cent during the periods of sitosterol administration. The drop was stated to be significant at the 5 per cent level or better in all but one subject. The greatest falls occurred again in the individuals with the highest initial cholesterol levels. The reductions in serum cholesterol appeared to be maintained during continued beta sitosterol administration. Confirmation of these observations was made by J. M. Lesesne, C. W. Castor, and S. W. Hoobler (*Univ. Mich. Med. Bull.* **21,** *13* (*1955*)). In this study 4 patients received beta sitosterol and 3 patients were given soybean sterols. The dose in both instances amounted to 3 g. before each meal. A significant decrease (from 10.8 to 22.5 per cent) in the serum total cholesterol was noted during the treatment period which extended for three to eight months.

M. M. Best, C. H. Duncan, E. J. Van Loon, and J. D. Wathen (*Am. J. Med.* **19,***61* (*1955*)) have extended their observations with beta sitosterol to include a total of 14 subjects. In this publication the dose is stated to be 6 to 8 g. before each meal, with a total daily dose of between 20 to 25 g. The previously observed decline in total serum cholesterol was again confirmed in these patients. Of considerable interest is the observation that serum total lipid, serum neutral fat and to a lesser extent serum lipid phosphorus declined during the periods of sitosterol ingestion. Ultracentrifugal studies of serum lipoproteins were interpreted as showing a "trend" toward lowering of the S_f 3 to 10, 10 to 30, and 30 to 100 components. It was suggested by the authors that the changes in the other lipids were in some fashion compensatory to the lowering of cholesterol values. The possibility that beta sitosterol might interfere with fat absorption was also considered. No gross changes in body weight were noted during the treatment with sterol. However, measurements of dietary intake were not made and possible fecal losses of fats were not studied. Carefully controlled metabolic studies will be required to clarify the effect, if any, of beta sitosterol on fat metabolism.

The findings of C. F. Wilkinson, E. Boyle, R. S. Jackson, and M. R. Benjamin (*Metabolism* **4,** *302* (*1955*)) are at variance with the work summarized above. Studies were carried out on 7 subjects, 4 of whom had arteriosclerotic heart disease with relatively normal cholesterol values. Two subjects had essential xanthomatosis and one had hypercholesterolemia with nephrosis. These patients were studied under hospital conditions during a control period of three weeks when a fat-free diet was ingested. An unimpressive lowering of the serum total cholesterol occurred. During the next phase of the study 49 g. of vegetable fat was added in place of an isocaloric amount of carbohydrate. A restoration of the cholesterol level toward normal appeared to take place on this diet. During the third phase of the study "sitosterol" was given as a soy sterol concentrate to 4 of the original 7 subjects. The principal sterol in the preparation used is stated to be gamma sitosterol and the daily dose was between 5 and 10 g. of sterol. No significant change in the serum cholesterol levels was noted during the period when the sterol was taken along with the vegetable fat diet. Even when these subjects were allowed to have an unlimited diet no change in cholesterol was apparent during a period of two to fourteen weeks of observation while taking "15 to 30" g. a day.

The failure of Wilkinson *et al.* to achieve reductions in the total plasma cholesterol with sitosterol feeding is difficult to reconcile with the experience of others. The daily dose of active sterol administered is difficult for the reader to determine and may have been less than that used by the other investigators. Moreover, the principal sterol used, gamma sitosterol, may be less effective in inhibiting the intestinal absorption of cholesterol than beta sitosterol. It may also be significant that 3 of the 4 subjects studied were not hypercholesterolemic and large

reductions of plasma cholesterol could not be anticipated in such individuals. The fourth subject was suffering from nephrosis which might represent a special problem.

The eventual usefulness of beta sitosterol in controlling hypercholesterolemia cannot be predicted with confidence on the basis of the present evidence. It would seem that modest reductions in the serum cholesterol of the order of 5 to 25 per cent can be obtained with the administration of relatively large amounts of sitosterol before each meal. The physical characteristics of the preparations thus far available will make patient acceptance difficult owing to their somewhat unpleasant chalky nature. These compounds may act not merely by blocking the absorption of dietary cholesterol but may also inhibit the reabsorption of biliary cholesterol. Whereas it may be possible to show that these substances do in fact lower the total serum cholesterol, it will be extremely difficult to establish whether the relatively small drops that many individuals manifest are of significance in the prevention or treatment of human atherosclerosis. Further developments in this field will be of interest.

ELECTROLYTE LOSSES FROM SWEAT IN FIBROCYSTIC DISEASE

Two cases of heat exhaustion occurring in children with fibrocystic disease have been reported by S. J. Harris and R. L. Lester (*New Engl. J. Med.* **253,** *223 (1955)*). Both of these children were admitted during a period of hot weather. They had both been anorexic and vomiting for three and four days and were severely dehydrated and febrile on entry. Study of electrolytes in serum drawn soon after therapy was started disclosed low levels of sodium, chloride and potassium. Although treatment was instituted promptly, one child died.

The recognition and analysis of this syndrome is of recent origin. Five years ago W. R. Kessler and D. H. Andersen (*Pediatrics* **8,** *648 (1951)*) pointed out that of 10 children treated for heat exhaustion, 5 had fibrocystic disease. As fibrocystic disease was known to involve exocrine glands at many sites throughout the body, a study of the secretion of the sweat glands was made. Observations by R. C. Darling, P. A. di Sant'Agnese, G. A. Perera, and Andersen (*Am. J. Med. Sci.* **225,** *67 (1953)*) demonstrated that children with fibrocystic disease had increased concentrations of sodium and chloride in sweat. This sweat was collected from the abdominal wall by means of a gauze pad when the air temperature was 89° F. and the humidity was 50 per cent.

Subsequent detailed studies by P. A. di Sant'Agnese, R. C. Darling, G. A. Perera, and E. Shea (*Pediatrics* **12,** *549 (1953)*) confirmed these observations and clarified the defect. In children with fibrocystic disease, the average sweat sodium, chloride and potassium concentrations were 133, 106 and 18 milliequivalents (mEq) per liter respectively. These were compared to the sweat gland secretions of the normal child in whom the sodium, chloride and potassium concentrations were 59, 32 and 12 mEq per liter respectively. All of these differences were highly significant. The volume of sweat was unaltered in those with fibrocystic disease.

These workers then studied four groups of children with fibrocystic disease. The first group consisted of children with complete pancreatic insufficiency, and moderate to severe pulmonary involvement. The second group had complete pancreatic insufficiency without respiratory involvement. The third group showed partial or no pancreatic deficiencies and moderate to severe pulmonary disease; the fourth demonstrated only pulmonary disease. There were no intergroup differences in the concentra-

tions of sodium, chloride, or potassium in the sweat, thus implying that the defect in the electrolyte concentration of sweat is part of the disease and not a result of involvement of other organs by the disease. This was further substantiated by examination of the sweat secretions from a group of patients without cystic fibrosis and with comparably severe pancreatic or pulmonary disease. In these cases the sweat electrolytes were normal.

It is known that the sweat sodium concentration will markedly decrease in response to a low sodium intake or administration of ACTH or desoxycorticosterone. When a patient with fibrocystic disease was placed on a low sodium diet the urinary sodium decreased in the expected manner, but the sweat sodium showed only a relatively small drop. The response to ACTH was similar, the kidney responding normally with respect to sodium excretion, and the sweat sodium dropping but 30 mEq to a concentration still well above normal. Thus, this syndrome seems to be clearly outlined as another example of end-organ failure—in this case an inability of the sweat glands to abstract sodium from their secretions. The clinical picture, then, becomes straightforward. With hot weather there is increased perspiration and increased salt loss. If salt intake fails to keep up with the loss the classic sodium depletion syndrome ensues, with loss of extracellular fluid, dehydration, vomiting and collapse. The prophylactic measure of a liberal or supplemented salt intake during the hot weather should prevent the occurrence of this syndrome.

Harris and Lester (*loc. cit.*) suggested that potassium should also be employed prophylactically. This suggestion was made on the basis of the established extra loss of potassium in the sweat and the low potassium in sweat noted in both patients studied. However, the extra sweat potassium loss of 6 mEq per liter can scarcely be important when food intake is maintained. Furthermore, a low serum potassium following vomiting, and in one case after sodium lactate administration, is easily explained by known mechanisms, as pointed out by the authors. It would seem that prophylactic potassium administration is unnecessary in these children when sodium balance and food intake are maintained. Potassium replacement is, of course, a necessary adjunct to therapy when external loss is increased by vomiting and developing alkalosis.

PHENYLPYRUVIC OLIGOPHRENIA

Dietary constituents may be implicated in the etiology of human disease in two general ways. An essential nutrient may be present in the diet in insufficient amounts, or intestinal absorption may be impaired so that signs and symptoms of deficiency may develop. On the other hand, while it is well known that excessive ingestion of certain nutrients may be harmful, it is not so widely recognized that individuals may have "errors" of metabolism whereby even the "normal" intake of a particular nutrient may lead to human disease. Examples of such disease mechanisms are the accumulation of galactose in galactosemic infants, of copper in Wilson's disease (hepatolenticular degeneration) and the excessive storage of iron in hemochromatosis.

Recently some workers have suggested that the accumulation of phenylalanine and its metabolic products may contribute to the development of a form of mental deterioration in infants called phenylpyruvic oligophrenia. In addition to mental deterioration these infants exhibit dermatitis, fair hair, a musty smell, dental abnormalities and convulsive seizures. It is known that in this syndrome the conversion of phenylalanine to tyrosine is impaired, and phenylalanine accumulates in the blood and cerebrospinal fluid. Phenylalanine and its derivatives phenylpyruvate and phenyl-

lactate are excreted in the urine. The inborn "error" of metabolism does not involve the use of phenylalanine for the synthesis of liver, kidney, brain, or plasma proteins.

The effect of feeding diets restricted in phenylalanine on the clinical and biochemical abnormalities in phenylketonuric patients has been reported from two clinics, H. Bickel, J. Gerrard, and E. M. Hickmans (*Acta paediat.* **43,** *64* (*1954*)) working in Birmingham, England, and M. D. Armstrong and F. H. Tyler (*J. Clin. Invest.* **34,** *565* (*1955*)) from Salt Lake City. The British workers prepared a phenylalanine-free diet using a charcoal-treated casein hydrolysate as a source of amino acids. This diet was fed to a 2 year old girl suffering from phenylpyruvic oligophrenia. A rapid fall in the level of phenylalanine in the blood occurred and the excretion of phenylalanine and its derivatives in the urine decreased almost to normal. Because weight loss occurred and the level of tyrosine in the blood was unmeasurable, tyrosine, which had also been removed from the casein by charcoal, was restored to the diet with temporary weight stabilization. The improvement of the child on the special diet is difficult to evaluate. The hair and skin lesions improved and the musty odor disappeared. Some improvement in behavior was also noted over an eleven-month period. The addition of 4 to 5 g. of L-phenylalanine to the daily diet produced an exacerbation of her mental and behavioral deficiencies.

The report of Armstrong and Tyler describes observations on 5 children with phenylketonuria, while on a low phenylalanine diet. All the amino acids except phenylalanine, serine, proline and hydroxyproline were provided in the diets in synthetic form. The children ranged in age from 8 months to 4½ years at the start of the dietary study. They consumed the diet for various periods up to many months. As is characteristic of this disorder, the clinical condition of the individual patients varied greatly. Some degree of improvement in mental and motor performance was noted in all the children studied. The convulsive manifestations were most readily controlled by the low phenylalanine diet. It is significant that the most striking clinical result was observed in the youngest subject. An 8 month old boy had had almost continuous epileptiform seizures until he was given the restricted diet. Within three weeks convulsions had terminated and he has continued to develop normally on the diet. The brief readministration of phenylalanine to this boy produced only equivocal worsening of his condition.

The biochemical changes which Armstrong and Tyler observed confirmed the findings of Bickel *et al.*

These studies have not definitely established the identity of a neurotoxic metabolite of phenylalanine, and Armstrong and Tyler suggest that "it seems probable that unknown and possibly complex features other than the known defect in phenylalanine oxidation operate in conjunction with it and contribute to the mental defect." While the clinical response of some of the patients studied has been equivocal and disappointing, the possibility is suggested that earlier recognition and dietary therapy beginning in infancy may result in substantial benefit. At present the practical obstacles to the long-continued ingestion of phenylalanine-poor diets seem large, but they could be overcome if these individuals could thereby be salvaged for a useful life.

NITROGEN BALANCE STUDIES AFTER TUBE FEEDING IN CANCER CACHEXIA

It has been an undocumented clinical impression that cancer patients are unable to utilize protein in the same manner as non-cancer patients. The over-all economy of nitrogen and calories in patients with terminal cancer has been the subject of

metabolic studies, most of which tend to give quite a different answer. M. D. Pareira, E. J. Conrad, W. Hicks, and R. Elman (*Cancer* **8,** *803 (1955)*) have examined the changes in nitrogen balance, body weight, plasma proteins and hemoglobin levels after the tube feeding of a 3500-calorie, 210-gram protein diet to 10 patients with terminal cancer. This diet was composed of 900 g. of a dry mix containing powdered whole milk, nonfat milk solids, calcium caseinate, dextrose and dextrimaltose with added vitamins and minerals. The mixture was given suspended in 1.8 to 2.4 liters of water.

Gastrointestinal tolerance to this diet was good, mild diarrhea occurring in 7 per cent of all patients. The diarrhea responded in most cases to slowing of the feeding rate. There was no sensation of fullness, and normal satiety was achieved with intermittent feedings. Apart from the balance studies, a most significant finding was the return of appetite in previously malnourished anorexic patients. This, in itself, led to rehabilitation in some instances, although the effect on life expectancy could not be gauged.

All 10 cancer patients exhibited a weight gain after tube hyperalimentation for periods averaging twenty-four days. The theoretic weight gain, calculated from nitrogen retention, in most instances agreed well with the actual weight gain. The less than perfect agreement is easily understandable because, as pointed out by Co Tui *et al.* (*Surg. Clin. North America* **29,** *449 (1949)*), unpredictable changes in extracellular fluid make difficult the comparison of actual and theoretic weight gain on the basis of nitrogen balance alone. Along with the weight gain noted, all patients also showed a rise in hemoglobin and 5 showed a rise in serum albumin. The failure of the serum albumin to rise concomitantly with the positive nitrogen balance and gain in hemoglobin may reflect the previous considerable depletion in protein stores.

Tentative conclusions from this study were that cancer cachexia was due solely to malnutrition sustained by anorexia, and that hyperalimentation could reverse not only changes in body weight but in appetite as well.

Similar conclusions have been reached by Co Tui and co-workers (*loc. cit.*), who also studied protein nutrition in a group of patients with cancer. When these patients were presented with a diet of their own choosing, nitrogen equilibrium was not obtained. When an attempt was made to force an increased intake of calories and nitrogen, significantly positive nitrogen balance was rarely obtained. Supplementation with protein hydrolysates led consistently to positive nitrogen balances, demonstrating that the anorexia and malnutrition of cancer patients can be circumvented.

Certain confusion has arisen in regard to this type of study because of the occasional findings of positive nitrogen balance in the presence of caloric deficit. This implies that the positive nitrogen balance was due to tumor growth only and raises the question of whether this conclusion would not be valid when positive caloric balance is achieved. A careful study in this regard was performed by C. Waterhouse, L. D. Fenninger, and E. H. Keutmann (*Cancer* **4,** *500 (1951)*), who analyzed caloric intakes and expenditures in 8 patients with rapidly progressive cancer. Four patients, 3 of whom had lymphomas, showed positive nitrogen balance in the presence of negative caloric balance. It is suggested from this that cancer tissue could "trap" nitrogen, although body proteins might be breaking down at an accelerated rate. These workers also noted that a positive caloric balance could be obtained if caloric intake was sufficiently high. They state that positive nitrogen balances were always seen in patients with progressive cancer, even in the presence of caloric deficit.

The conflict between these findings and the consistently negative nitrogen balances noted in the first two papers can be rec-

onciled by examining the types of cancer worked with by each group. In the presence of relatively slowly growing cancers, nitrogen accretion by cancer, per se, makes little contribution towards offsetting the negative nitrogen balance due to decreased food intake. However, when the mass of a cancer is increasing rapidly, as in lymphomas, positive nitrogen balance due to increasing tumor nitrogen may conceal the breakdown of body protein.

From these studies, it would seem that the promotion of a positive caloric and nitrogen balance is clinically of benefit to the patient with terminal cancer. This conclusion, of course, ignores any possible detrimental effect from enhancement of cancer growth as a result of augmented protein intake. Positive caloric balance and increase of body protein can be achieved in those malnourished patients with extensive metastatic cancer.

WEIGHT STANDARDS

Body weight is a limited criterion of nutriture (J. Brozek, *Am. J. Phys. Anthropol.* **11,** *147* (*1953*)). Nevertheless, the comparison of an individual's actual weight with a proper reference weight will always remain a starting point in the evaluation of nutritional status. The recent developments in the field of weight standards have been discussed in this journal on several occasions (*see, e.g., Nutrition Reviews* **11,** *229, 259* (*1953*)).

In the United States the overwhelming majority of the printed tables of standard weights go back to the Medico-actuarial tables of 1912, published by the Association of Life Insurance Medical Directors and the Actuarial Society of America. In spite of many uncertainties (secular changes in height-weight relationships, correction for clothing and shoes, inadequacy of height as the only criterion of bony development and neglect of the lateral aspect of body frame), these tables have served as reference standards for almost fifty years and have been used in other parts of the world as well. For purposes of description and comparison of different samples, one is on reasonably safe grounds in using these data. For purposes of evaluation, unresolved questions such as the representativeness of the samples on which the 1912 tables were based and their present validity become distressing.

In order to construct tables of average weights for different heights, which could be used with some confidence for the Canadian population, the Nutritional Division of the Department of National Health and Welfare, Ottawa, in 1953 carried out a large-scale survey (L. B. Pett, *Canad. Med. Assn. J.* **72,** *12* (*1955*)). This undertaking is of interest on several counts, not the least of which is the fact that it resulted in the first representative table of weights and heights ever obtained in Canada and one of the first in the world. It provides a clearly defined baseline for the studies of overweight as a health hazard and a reliable reference standard for physicians and nutritionists. While the questions concerning the relationships among "average," "normal," or "ideal" weights can not be solved without additional medico-actuarial information, the data obtained from the survey make possible a sound comparison of a Canadian's weight with the average weight of other Canadians in the same height and age group.

A noteworthy feature of the survey was a careful sampling of the Canadian population of 15,000,000 people, carried out with the assistance of the Dominion Bureau of Statistics. The stratified sample was obtained by measuring people in 59 regional units of Canada. In each area the specified number of households was selected at random from lists of census visitation records. Excluded from the sample were individuals confined to bed through illness,

pregnant women, children under 2 years, members of the armed services in camps or barracks, Indians on reservations, and certain classes of cripples and invalids. About 20 per cent of persons who were scheduled to be measured could not be reached. This is a very satisfactory response rate and allowance was made for the "misses" in the original sampling plans. The total sample included close to 22,000 people. A real effort was made to choose the regions and individuals so as to obtain average weights representative of the Canadian population to an accuracy of 5 per cent.

In the paper cited, average weights are given for males and females between ages 15 and 65, heights 4 ft. 11 in. to 6 ft. 3 in., and 4 ft. 8 in. to 5 ft. 11 in., respectively. The subjects were weighed and measured "in ordinary indoor clothing, without shoes." It is hoped that estimates of the corrections needed to transform these data into values obtained for individuals in the nude will be provided. It is noted that at younger ages the average (mean) weights at a given height are probably close to the "ideal" weight, with 10 per cent above or below the tabular values to be allowed for different body types. After the age of 30 it is considered advantageous to keep the body weight somewhat below the tabular average.

The values are given in the tables as actual averages. This is, unquestionably, the basic information and it is hoped that in the final publication the number of individuals, on which each mean is based, will be indicated. However, for actual use of the tables it will be necessary to smooth the age trends. Thus it is very probable that the fact that tall women (5 ft. 11 in.) in the age bracket of 35 to 44 years are lighter (mean weight of 147 pounds) than those 30 to 34 years old (mean weight of 150 pounds) reflects simply fluctuations due to small sizes of the samples in these categories.

In a companion report (L. B. Pett, *Am. J. Pub. Health* **45,** *862 (1955)*) the age trend of average heights and weights are given, by age and sex, in tabular and graphic form, for ages 2 to 65 years and over. At the age of 12 and 13 the girls are both taller (58.2, 60.4 in.) and heavier (92 pounds, 102 pounds) than the boys (57.4 and 59.3 in.; 84 and 94 pounds), indicating an earlier physical maturing. In height there is a parallel decline in the two sexes, continuing from the fourth decade of life onwards. In weight, Canadian men reach the peak in the years 30 to 44 while women continue to gain weight through the period of 55 to 64 years.

The new Canadian averages for height and weight are compared in a tabular form with the results of some other studies made in Canada and abroad. While the rate at which adult height is reached has increased in the past thirty years, the children being taller (and heavier) than their parents at the same age, final adult heights seem not to have changed much over the years.

It was realized that overweight is not the same thing as obesity, the latter referring to an excessive accumulation of body fat. Under certain conditions such accumulations may occur without much (or any) weight increase and means are needed for a physiologic interpretation of body weight in terms of body composition. As a criterion of leaness-fatness, skinfold measurements were included, and the results will be published in a separate communication.

The body weight and chest girths of Scotsmen have been studied in relation to stature, age and social status by E. M. B. Clements and K. G. Pickett (*Brit. J. Prevent. Social Med.* **8,** *99, 108 (1954)*). The values were derived from a sample of records of medical examinations of men called up for military service in 1941, with the subjects wearing trousers only while they were weighed. It may be useful to note that the skewed distributions of body weights are normalized when the values are expressed as the logarithms of the measurements made in pounds. Such a trans-

formation facilitates the use of statistics assuming a normal distribution of the variates.

The following are the principal findings: (1) the mean weights in the different social classes do not differ significantly; the apparent differences between the mean weights are mainly accounted for by differences in stature. (2) The men regarded as fit for military service weighed 4 pounds more, on the average, than the unfit men. (3) The different age trends present at the extremes of weight distribution are pointed out, with the mean values for the first percentile of 104 pounds at age 18 to 19 and no increment with age (102 pounds at age 36 to 37 years) as contrasted with 174.4 and 189.4 pounds corresponding to the 99th centile at these two ages.

It should be stressed that chest girth, by itself, is not a useful measurement for purposes of nutritional anthropometry. Biologically, it is a complex number. It has been used, in the past, in computing indices of "nutritional status" while at other times it was employed as a means of classifying men into physical "types" which, in the eyes of some workers, should be independent of variations in nutritional status. Thus variations in nutriture and in the "constitutional" aspects of body build have been frequently confused. While a simple anthropometric separation of the bony and muscular component in the chest girth is not feasible, caliper measurements of skinfolds or roentgenographic evaluation of the layer of skin plus subcutaneous fat make possible the evaluation of the contribution of the fat layer. Preferably, the measurements should be made at the same level at which the tape is applied. Skinfolds may be made both on the side of the chest cage (over the ribs, along the midaxillary line), and at the back (at the lower angle of the scapula).

VITAMIN B_{12} AND SKIN DEPOSITS OF CHOLESTEROL AND LIPID

In a preliminary study R. C. V. Robinson (*J. Invest. Dermat.* **24,** *111 (1955)*) presents some stimulating evidence concerning the effects of vitamin B_{12} on xanthelasma palpebrarum. The small yellow tumors of the eyelids which bear this name have long been associated with disturbed lipid metabolism, specifically cholesterol metabolism. They are thought to be one of the common superficial signs of hypercholesterolemia, particularly when it is associated with the generalized lipoidosis known as essential xanthomatosis. They are also known to be common in diabetic subjects, and Robinson's observations suggest that this is particularly true of diabetics of the Jewish race.

Reasoning from recent evidence that vitamin B_{12} exerts a singularly important role in carbohydrate and lipid metabolism (C. T. Ling and B. F. Chow, *J. Biol. Chem.* **198,** *439 (1952)*; **206,** *797 (1954)*), Robinson decided to ascertain the effects of vitamin B_{12} therapy on this external manifestation of disturbed lipid metabolism. This was prompted in part by the fact that no suggested therapeutic agent for this lesion has been very successful in the past.

Several interesting observations were made in this study. Although in no case did the lesions disappear entirely, 35 patients treated with 30 to 1000 micrograms of vitamin B_{12} parenterally once a week over a six-week period showed regression of the lesions characterized as "good" in 26 instances and excellent in 5. On the other hand, of 30 patients treated with 1000 micrograms of vitamin B_{12} orally per day for thirty days only 5 improved while 25 showed no change. The failure of the oral route may be related to the fact that this group of patients apparently all had much higher serum cholesterol values than the groups treated parenterally. Absorption

studies on individuals of the group treated orally revealed that the drug was being absorbed.

That vitamin B_{12}-induced improvement in the lesions was not due to simple lowering of the serum cholesterol levels was indicated by the fact that there was no downward trend in the average cholesterol concentrations when these were determined at weekly intervals during therapy.

It is unfortunate that it was not possible to obtain biopsies before and after therapy in some of these patients. It may be that the flattening of the lesions observed by Robinson is essentially due to decrease in lipid stored by histiocytes in the corium. The part of the lesion which did not respond may be a minor, nonspecific fibrous connective tissue response to the stored lipid. In any case the relation of vitamin B_{12} to lipid metabolism would seem to merit further study. Factors which influence lipid metabolism in general may also have an effect on these xanthomatous lesions of the skin. This is indicated by the recent observation that heparin therapy may benefit the xanthomatous skin lesions in idiopathic hyperlipemia (W. F. Lever, F. S. M. Herbst, and N. A. Hurley, *Arch. Dermat.* **71,** *150 (1955)*; T. Cornbleet, *Ibid.* **71,** *172 (1955)*). Whether abnormal deposits of cholesterol in skin and artery respond in a similar fashion when vitamin B_{12} is administered remains to be determined.

FOOD INTAKE AND ENERGY EXPENDITURE

At the turn of the century, the classic work of Atwater and his colleagues with the "human calorimeter" stimulated great interest and much activity in problems of food intake and energy expenditure, but with the coming of the age of vitamins and minerals there was a shift of interest and emphasis, and it took a major human conflict in the shape of World War II, with its attendant problems of food scarcity and the need for rationing, to refocus the interest of nutritionists on the ubiquitous calorie (*see, for example, Nutrition Reviews* **9,** *40 (1951)*; **12,** *196 (1954)*).

The problem of the varied energy demands of different occupations has long been of interest to the calorie-conscious nutritionist, and a recent report of the British Medical Research Council (R. C. Garry, R. Passmore, G. M. Warnock, and J. V. G. A. Durnin, *Med. Res. Council (London) Special Report Series No. 289 (1955)*) on the energy expenditure and food intake of coal miners and clerks provides much useful information, and is a good model for future investigations of this nature. A survey was made of the energy expenditure and food intake for one week of 19 underground miners and 10 clerks at a Scottish coal mine. The food intake of the subjects during the one-week observation period was determined by a dietary survey employing the individual inventory method. Energy expenditure was determined by indirect calorimetry, and the Kofrányi-Michaelis (E. Kofrányi and H. F. Michaelis, *Arbeitsphysiol.* **11,** *148 (1940)*) respirometer was used for this purpose. The value of this machine, which has been much used in Europe, particularly in Germany, has already been discussed (*Nutrition Reviews* **12,** *196 (1954)*). It is light (3 kg.), and is essentially a portable gas meter which directly measures the volume of expired air and simultaneously retains a sample for later analysis. Further, the energy expenditure, measured in calories per minute during diverse occupations and activities, may be readily studied, as the wearer is slightly, if at all, hampered in the performance of muscular work.

The total average daily expenditure of

energy by the underground miners was 3660 calories, while that of the more sedentary clerks was 2800 calories. This difference in energy expenditure was due to the additional demands on energy made by the miners during their working hours. Both groups showed much the same expenditure of energy at rest, off duty and at home. Interestingly enough, the miners spent 38 per cent of their time underground in the mine sitting about, and although the metabolic cost of the various mining activities (for example, hewing, loading and timbering) was about 7 calories per minute, the mean rate of energy expenditure during the entire time spent at work underground was only 4.3 calories per minute. Numerous interruptions and the time spent getting to and from the coal-face accounted for this difference, and not failure of the miners to work hard.

The idea that manual laborers need more food than sedentary workers has long been known, and in the present study it is not surprising to find that the miners took in some 990 calories more per day than the clerks. The miners ate more of nearly all foods. Further, they ate more main meals and snacks on working days and fewer snacks on nonworking days than did the clerks. However, there was no indication that carbohydrate provided the additional energy needed by the miners, the percentages of the calories derived from protein, fat and carbohydrate being essentially the same in the two groups of workers. The diets of both groups were assessed in accordance with recommendations suggested by the British Medical Association (*British Medical Association (London) Report of the Committee on Nutrition (1950)*); and only in respect to riboflavin, which was slightly below the recommended allowance, was the average diet other than adequate.

There is still need for similar studies of people of other occupational groups, although much has already been accomplished in this field. H. Kraut and H. Bramsel (*Arbeitsphysiol.* **12,** *197 (1942)*; *see also Nutrition Reviews* **9,** *273 (1951)*), for example, prepared a comprehensive table of estimated caloric requirements for 150 jobs in agriculture, industry, transportation and business, and G. Lehmann, E. A. Müller, and H. Spitzer (*Arbeitsphysiol.* **14,** *166 (1950)*) reported a study comprising some 1800 determinations of energy expenditure made on a total of 437 industrial and 167 different activities. Even the energy expenditure of the housewife has not escaped attention (W. Droese, E. Kofrányi, Kraut, and L. Wildemann, *Ibid.* **14,** *63 (1949)*). Much of the data in the textbooks pertaining to the energy demands of different occupations should be revised in the light of the studies mentioned above. Not only were the old methods (*e.g.*, the "human calorimeter" and the Douglas bag) somewhat unwieldy and cumbersome, and hence possibly inaccurate in assessing energy expenditure in muscular work, but the energy demands of many occupations may well have changed with the use of labor-saving devices that have been developed in recent years.

Apart from the obvious value of such studies in assessing the food requirements of a worker so that he can be nutritionally well equipped to withstand the energy expenditure demanded by his job, certain philosophic points also emerge. For example, J. C. D. Hutchinson (*Brit. J. Nutrition* **1,** *231 (1947)*), in a paper on the nutrition of domestic rabbits, has pointed out that although the available energy obtained from fattening a single rabbit by feeding it weeds was 549 calories, the available energy expended in collecting weeds to feed such an animal was 1022 calories! Presumably the more we find out about the energy demands made by different occupations and activities the less likely will we be to get involved in similar unfortunate and uneconomic situations.

TRYPTOPHAN REQUIREMENT OF THE WEANLING PIG

In the past few years temporary values have become available for the quantitative requirements for most of the essential amino acids for swine (*Nutrition Reviews* **9,** *220 (1951)*; **12,** *68 (1954)*; **13,** *304 (1955)*). With this information, knowledge of the nutritional requirements of swine has progressed to the stage previously reached for the rat, mouse, chicken and adult man.

Earlier studies indicated that the weanling pig may require 0.15 to 0.2 per cent of tryptophan in the diet (*Nutrition Reviews, loc. cit.*). Also, the Committee on Animal Nutrition ("*Nutrient Requirements for Swine,*" *National Academy of Sciences—National Research Council, Publication No. 295, Washington, D. C. (1953)*) suggested that 0.2 per cent of tryptophan is required by weanling pigs. More recent studies suggest that these values may be too high.

D. E. Becker *et al.* (*J. Animal Sci.* **14,** *664 (1955)*) have studied the tryptophan requirement of weanling pigs. They used a total of 96 animals each weighing about 30 pounds at the start of the study, which consisted of 3 experiments. The pigs were reared in individual wire-bottom cages for the four-week experimental periods. The practical rations, low in tryptophan, consisted chiefly of yellow corn (80 to 88 per cent) and menhaden fish meal (9.8 to 12.7 per cent) supplemented with adequate levels of minerals and vitamins. The protein levels of the diets varied from 15.3 to 19.6 per cent and contained, according to the authors, only 0.075 per cent of tryptophan. A level of 36 mg. of niacin was calculated to be present per kilogram of ration, which is more than adequate for the pig.

The growth results indicated that the pig required no more than 0.115 per cent of L-tryptophan in a diet containing 15.3 per cent of protein. Adding 5 per cent of zein to the diet, which raised the calculated protein level to 19.3 per cent, did not raise the tryptophan requirement. Also, the authors reported that the pig appeared to utilize a considerable share of the D-isomer of tryptophan when it was fed in a racemic mixture, which is similar to the utilization of tryptophan by the rat. There was no tendency for the DL-tryptophan to depress growth, contrary to the results obtained in less convincing studies on this point from the same laboratory by D. E. Becker, M. G. Nesheim, S. W. Terrill, and A. H. Jensen (*J. Animal Sci.* **14,** *642 (1955)*).

No effect of tryptophan deficiency was seen on carcass yield or specific gravity of the carcass. However, it was found that the thyroid gland was significantly larger in the tryptophan-deficient pigs. The reason for this was not readily apparent. Thyroid weight was not influenced by the feeding of an antibiotic, nor was there any significant effect of antibiotics on the tryptophan requirement, as has been reported in rats (*Nutrition Reviews* **12,** *152 (1954)*). However, the well known effects of antibiotics in increasing body weight gains and food efficiency were seen when they were added to rations either low or high in tryptophan.

Although the authors state that their results do not "conform with the idea that the tryptophan requirement will be higher with a higher protein diet," they point out that they have only studied a relatively narrow range of protein levels. This is especially true since the zein which they used is only 44.8 per cent digestible (Becker and coworkers, *J. Animal Sci.* **14,** *642 (1955)*). Thus, in effect, the range of available protein levels tested was from 15.3 to only about 17.6 per cent. Greater range of protein levels than this would have to be used to determine adequately whether the level of protein influenced the tryptophan requirement.

A major weakness of this study should be pointed out in order to evaluate properly the conclusions. The level of tryptophan chosen as the requirement (0.115 per cent) is

based not only on the growth results obtained by adding various levels of tryptophan to the ration but also on the tryptophan content of the basal ration as "determined by assay." Unfortunately, no mention is made of the type of assay used or of its accuracy and specificity. It is reasonably possible that a ration containing 80 per cent of corn and 12.7 per cent of fish meal might contain more than the indicated 0.075 per cent of tryptophan. In fact, according to values published by the Committee on Animal Nutrition (*loc. cit.*), such a mixture may be calculated to contain 0.120 per cent of tryptophan, thus raising the requirement to 0.16 per cent, a value which agrees more with the requirements of other animals (*Nutrition Reviews* **8,** *248 (1950)*; **10,** *241 (1952)*).

In spite of the weaknesses which have been pointed out, these studies indicate that the pig has a relatively low requirement for tryptophan in the presence of ample niacin. These studies may stimulate additional work with swine and with other animals to estimate more accurately the tryptophan requirement and to study factors which affect the requirement.

UNIDENTIFIED MINERALS IN POULTRY NUTRITION

Renewed interest has recently developed in the field of unidentified mineral requirements for the growth of poultry. It is becoming increasingly evident that at least one unidentified mineral (*i.e.*, not yet recognized as essential) may be needed in the diet of the chick for maximum growth. It is likely that the mineral, or minerals, when identified, will spare, or substitute for, previously suggested unidentified growth factors (*Nutrition Reviews* **8,** *298, 301 (1950)*; **10,** *178 (1952)*).

A. B. Morrison, M. L. Scott, and L. C. Norris (*Poultry Sci.* **34,** *738 (1955)*) and Norris (*Proc. Tenth Distillers Feed Conference, Cincinnati, Ohio, p. 39, March 3, 1955*) have studied the unidentified mineral requirements of specially depleted chicks, which were the offspring of hens fed diets low in unidentified growth factors. The basal purified diet fed the chicks contained, in percentages, glucose 61.3, soybean protein 25.47, fat 3, cellophane 3, methionine 0.7, glycine 0.3, and supplements of minerals and vitamins at levels equal to, or higher than, recommended levels.

The addition of 2 per cent of the ash of a mixture of various crude feedstuffs (prepared in a muffle furnace at 525° C. for four hours) gave a marked response in growth (body weight rose from an average of 336 g. to 407 g. in four weeks). Additions to the diet of aluminum, barium, boron, bismuth, bromine, chromium, fluorine, molybdenum, or vanadium were ineffective in substituting for the ash. Increasing the level of minerals known to be needed was also ineffective, so it was believed that the active component was a mineral not yet recognized as essential. Since a high level of ash was used (2 per cent) it is possible that a change in mineral balance had affected growth.

A mixture of untreated feedstuffs as a source of unidentified organic factors gave a further response in growth (37 g. at four weeks) when added to the basal diet. However, no evidence was given for the existence of more than one organic growth factor in such products as fish solubles, dried whey, forage concentrate, penicillin mycelium meal, and distiller's dried solubles.

Several details of experimental procedure are omitted from the papers describing the work of Norris and co-workers. No mention is made of the state of purity of the minerals used in the salt mixture, or whether distilled water was used for drinking instead of tap water. Also, it would be important to know if the chicks were raised in cages of

galvanized metal, in floor pens, or in glass- or plastic-lined cages. Such information would be helpful to evaluate the work properly. Final proof that the factor, or factors, is inorganic must await further evidence since, as Norris (*loc. cit.*) states, "although seemingly remote, the possibility exists that the growth-promoting factor in the ash is an organic or mineral-organic compound highly resistant to destruction at 525° C."

A similar growth response in depleted New Hampshire chicks was obtained from the addition of the ash of distiller's solubles (equivalent to a diet level of 3 per cent) to a highly purified diet in studies by J. R. Couch *et al.* (*Proc. Tenth Distillers Feed Conference, Cincinnati, Ohio, p. 47, March 3, 1955*). Weights of chicks on the basal diet averaged 247 g. at four weeks, while the addition of the ash increased growth so that they weighed an average of 286 g. Although the authors state that the levels of minerals present in the basal diet meet the National Research Council's recommended levels, no details of the ingredients used in the mineral mixture are given.

It may be noted that in both of these studies "depleted" chicks, obtained from hens fed rations low in unidentified factors, were used. Whether such depletion is necessary to obtain the growth effect with the ash is not known. In any event, it is reasonable to expect that the identity of the mineral, or minerals, responsible for the growth effect will be determined in the near future. Such reports will be awaited with interest.

THE *IN VIVO* METABOLISM OF FRUCTOSE

Recent interest in hexoses other than glucose for dietary and parenteral administration has stimulated research on the mechanisms by which these substances are metabolized (*Nutrition Reviews* **12,** *154, 187, 227 (1954)*). H. G. Hers and T. Kusaka (*Biochim. et biophys. acta* **11,** *427 (1953)*) found that liver extracts metabolize fructose through fructose-1-phosphate (fructokinase and ATP) to glyceraldehyde and dihydroxyacetone phosphate. A triokinase phosphorylates glyceraldehyde to glyceraldehyde phosphate. Further reactions of the triose phosphates follow the glycolytic scheme—condensation to hexose diphosphate and then formation of glucose and glycogen or conversion to pyruvate for subsequent metabolism to fatty acids, carbon dioxide, etc.

The rapid conversion of fructose to glucose by liver slices (S. S. Chernick, I. L. Chaikoff, and S. Abraham, *J. Biol. Chem.* **193,** *793 (1951)*) would be accomplished, according to this scheme, by first breaking fructose into two trioses. The trioses then recombine to form glucose-6-phosphate which is readily cleaved by glucose-6-phosphatase to glucose and phosphate. The question to be resolved was whether this seemingly complicated series of reactions represented the primary course of fructose metabolism in the body. In a recent paper H. G. Hers (*Ibid.* **214,** *373 (1955)*) has shown that his previous *in vitro* scheme does in fact hold for the liver *in situ*.

Fructose, glucose and sorbitol labeled in the 1 position with C^{14} were injected into rats and the distribution of C^{14} in liver and muscle glycogen was determined after one and three hours. Results for liver and muscle were similar; with glucose-1-C^{14} almost all of the C^{14} was found in the C-1 of the glucose obtained from glycogen. In experiments with fructose and sorbitol only 55 to 60 per cent of the label was in the C-1 position and 30 per cent was in C-6. These results are consistent with the scheme presented above and also indicate that sorbitol is oxidized to fructose before being further metabolized.

The low affinity of the muscle enzymes for fructose probably resulted in the bulk of the

labeled fructose being converted to glucose in the liver and then the newly formed 1,6-labeled glucose was incorporated into muscle glycogen. This point was further clarified by incubating diaphragm with fructose-1-C^{14}. Two mechanisms appear to operate: a non-specific hexokinase that converts fructose to glycogen without cleavage (via fructose-6-phosphate) and a specific fructokinase similar to that described for liver. The first mechanism is sensitive to insulin and preferentially phosphorylates glucose. The specific fructokinase action of muscle appears to be very slow (5 to 6 per cent).

Hers has thus applied the conclusions drawn from his experiments with tissue extracts to explain *in vivo* experiments and in so doing has elucidated the major mechanisms by which fructose and sorbitol are used in the body.

PROTEIN SYNTHESIS AND THE DYNAMIC STATE

The acceptance of Schoenheimer's theories of the dynamic state of protein constituents in the cell has been almost unanimous (*see Nutrition Reviews* **13,** *237 (1955)*). However, a recent paper by D. S. Hogness, M. Cohn, and J. Monod (*Biochim. et biophys. acta* **16,** *99 (1955)*) presents some strong reasons for reconsideration of this problem.

These investigators have carefully analyzed the induced formation of beta-galactosidase in *E. coli*, using as an inducing agent methyl-beta-D-thiogalactoside, which is itself neither hydrolyzed nor metabolized by the bacteria. To study the precursor of the enzyme, the cell proteins were labeled by allowing the culture to grow to starvation in a medium containing a limiting amount of sulfur as labeled ammonium sulfate ($(NH_4)_2S^{35}O_4$). With the protein fraction labeled, the bacteria were placed in a medium containing the inducer and again a limiting amount of ammonium sulfate, this time unlabeled. After having once more multiplied to the starvation point, the cells were lysed and the beta-galactosidase was isolated by a four-step process involving (1) removal of nucleic acids, (2) fractionation with ammonium sulfate, (3) further fractionation by starch electrophoresis, (4) precipitation with a specific antiserum. The enzyme thus purified was essentially unlabeled (its S^{35} content being less than 0.8 per cent of total cellular S^{35}) and therefore it was presumed to be synthesized *de novo* and not from pre-existing S^{35}-labeled cellular proteins. Previous work by this group (J. Monod, A. M. Pappenheimer, and G. Cohen-Bazire, *Biochim. et biophys. acta* **9,** *648 (1952)*) had shown the existence of an immunologically similar protein. The possibility that it is a specific precursor of beta-galactosidase is now ruled out.

The stability of cellular proteins was also tested. Bacteria were grown in a medium containing the inducer and a limited amount of unlabeled sulfur. After reaching the point of sulfur starvation, the cells were then transferred to a medium containing S^{35}-labeled sulfate without inducer, and again grown to starvation. Analysis for beta-galactosidase showed the total amount to be unchanged, although the cellular mass had increased 900 per cent during the second phase of growth. It could be calculated that the rate of breakdown of the enzyme in the absence of inducer was less than 0.2 per cent of the rate of synthesis in the presence of inducer. A similar but more elaborate experiment estimated the rate of breakdown of beta-galactosidase in the presence of inducer at less than 5 per cent of the synthesis rate.

Thus, beta-galactosidase has been shown to be completely synthesized from newly made amino acids, as the amino acid pool in *E. coli* is negligible. The stability of the protein enzyme within the cells was great, with very little suggestion of amino acid exchange, even after the inducer had been removed. It

can be further concluded that the cellular proteins are irreversibly synthesized and exist in a static rather than dynamic state.

Partial confirmation of this work is available in a report by D. B. Cowie, E. T. Bolton, and M. K. Sands (*J. Bact.* **60,** *233 (1950)*), who studied various aspects of sulfur metabolism in *E. coli*. They also noted that the exchange of bound sulfur with environmental sulfur was of a low order of magnitude.

The conflict between these findings and the current dynamic concept of protein turnover is difficult to reconcile. Hogness *et al.* point out, however, that their bacterial population is essentially homogeneous, with all cells actively growing and no detectable cell lysis. This is quite different from mammalian tissue where cellular growth embraces the entire range from actively multiplying cells to dying cells. It is suggested that what has been interpreted as a turnover phenomenon due to intracellular degradation of proteins may have been merely an expression of the rate of cell destruction and replacement. If this be true, we should, perhaps, refer to the dynamic state of tissue rather than of proteins. This concept will be difficult to verify in mammalian tissue, but the possible ramifications for the biologic sciences are many.

RADIATION DAMAGE AND FAT DEFICIENCY

One of the consequences of essential fatty acid deficiency which has received the most attention in recent years has been the decreased resistance of animals in this state to the effects of radiation injury (*Nutrition Reviews* **9,** *200 (1951)*). Following the observation that rats on a fat-free diet showed increased susceptibility to radiation injury (A. L. S. Cheng, G. D. Kryder, L. Bergquist, and H. J. Deuel, *J. Nutrition* **48,** *161 (1952)*), Deuel, Cheng, Kryder, and M. E. Bingemann (*Science* **117,** *254 (1953)*) ascertained that the nutritional deficiency in these animals was actually one of essential fatty acid, since it could be counteracted with ethyl linoleate. In subsequent experiments (Cheng, M. Ryan, R. Alfin-Slater, and Deuel, *J. Nutrition* **52,** *637 (1954)*; *Nutrition Reviews* **13,** *25 (1955)*), the original observations were extended and amplified to include studies of the effect of varying doses of linoleate in correcting this deficiency.

In a recent paper (A. L. S. Cheng, T. M. Graham, R. B. Alfin-Slater, and H. J. Deuel, *J. Nutrition* **55,** *647 (1955)*), this group combines their observations on the radiation sensitivity of fat-deficient animals with previous work on the interrelationship of linoleic and linolenic acids. Previously, it had been found that although linolenate was inadequate for curing essential fatty acid deficiency symptoms, when it was administered in admixture with linoleate it apparently became fully as active as the latter (S. M. Greenberg, C. E. Calbert, E. E. Savage, and Deuel, *Ibid.* **41,** *473 (1950)*). In the present case, rats on a fat-free diet with or without supplemental amounts of methyl linoleate, linolenate, or both were given seven weekly doses of 200 roentgens each of x-irradiation. Mean survival time increased with increasing doses of linoleate to 80 mg. per day. Methyl linolenate had no beneficial effect, but 10 mg. each of the two esters was about as effective as 80 mg. of methyl linoleate.

A consideration of the nature of essential fatty acid deficiency makes likely the possibility that the action of the supplement in these cases, as in many others, is in the replacement of damaged tissues. In the absence of essential fatty acid, the body is unable to repair tissues damaged by radiation or by any other means. The interesting apparent potentiation of linolenate by linoleate evidently needs further study. It is possible that the former acts as a protective

agent for the latter in its passage into and throughout the body, thus effectively increasing the amount of linoleate to reach the reactive centers. If this should prove correct, an explanation would be on hand for the divergent results of many workers on the potency of slightly impure linolenate as an essential fatty acid.

UPTAKE AND RETENTION OF CALCIUM IN THE RAT

Use of the radioactive calcium isotope Ca^{45} during the past decade has resulted in rapidly increasing knowledge of the details of the metabolic activity of calcium (*cf. Nutrition Reviews* **13,** *163 (1955)*).

As part of a study of nutritional factors in bone and tooth formation, H. D. Cremer, W. Herr, and H. Spath (*Biochem. Z.* **322,** *212 (1951)*) used Ca^{45}-labeled calcium salts mixed with the diet and administered by stomach tube. They demonstrated that in rats the degree of utilization of calcium salts decreased in this order: lactate and chloride; phosphate and carbonate; oxalate. This sequence is the same as that of the relative water solubilities of the salts.

In a continuation of this research, R. Antoni and H. D. Cremer (*Biochem. Z.* **326,** *311 (1955)*) investigated the effect of vitamin D on the utilization of labeled calcium fed as the citrate-complex salt. The recovery of Ca^{45} in the bones and teeth was poorer when calcium was fed in this form than when fed as the lactate or chloride; recovery in the feces was greater. Utilization of the citrate was significantly increased by the addition of vitamin D to the mixture. This finding is, of course, consistent with the well established effects of vitamin D administration, but does not constitute evidence for or against the theory that a direct mode of action of the vitamin on calcification exists apart from its action on intestinal absorption.

When dry mixtures of tertiary calcium citrate and secondary calcium phosphate (both containing Ca^{45}) were administered with the food, the utilization of calcium as measured by radiocalcium uptake seemed to vary inversely with the amount of citrate fed. Assay of the bones and teeth of rats killed at various times after discontinuance of the diet containing Ca^{45} showed that the accumulation of radiocalcium in the tibias, mandibles and molars (expressed as per cent of administered activity) reached a maximum within twenty-four hours and then fell steadily. The molars lost activity at a slower rate than the bones. On the other hand, the incisors were reported to attain their maximum activity between the second and third week after termination of the radioisotope dosage. The authors speculated that since the rat incisors are in a state of continual growth they incorporate Ca^{45} as it is slowly released to the circulation by exchange from bone and other tissues in which it was initially deposited.

Unfortunately, the analytic data are not given in terms of specific activity, making it difficult to evaluate the importance of bone-salt accretion. Furthermore, the conclusion that the activity of the incisors continues to increase during the first two weeks is based on average values from only 2 animals at a single time interval. Further substantiation of these interesting observations would be desirable.

EFFECT OF FEEDING DIETS EXPOSED TO ETHYLENE OXIDE

Ethylene oxide has been used for many years as an insecticide and a bactericide. It has been used to some extent in the food industry (C. R. Phillips and S. Kaye, *Am.*

J. Hyg. **50,** *270 (1949)*). E. A. Hawk and O. Mickelsen (*Science* **121,** *442 (1955)*) have presented evidence that the use of ethylene oxide for the sterilization of foods might present certain nutritional hazards. These workers fed weanling and 10 week old rats a purified diet, and a diet of natural foodstuffs, that had been treated with ethylene oxide. They noted that such diets would not permit normal weight gains.

The diet treatment consisted of spreading 500 g. of diet on large Petri dishes to a depth of 1 cm. The dishes were then stacked in a 10-liter vessel (desiccator without desiccant) to permit free access of gaseous ethylene oxide to the diets. Ten ml. (8.84 g.) of liquid (cold) ethylene oxide were introduced into the vessel and the lid was replaced. After fumigation for six to twenty-four hours at room temperature, the Petri dishes were removed and exposed to air overnight to dissipate residual gaseous ethylene oxide.

The untreated purified diet, containing casein, sucrose, vegetable oil, salts and vitamins, permitted weanling rats to gain 120 g. in four weeks. The same diet treated with ethylene oxide by the above procedure for eighteen hours resulted in gains of 22 g. in two weeks followed by weight losses of 14 g. per rat during the subsequent two weeks. The weight loss was followed by death during the fifth week of feeding the treated diet.

Contrasted with average weight gains of 172 g. in eight weeks on a diet of natural feedstuffs (ground dog meal pellets), the same diet treated with ethylene oxide for six, twelve, eighteen and twenty-four hours resulted in weight gains of 120, 117, 86 and 57 g., respectively, in eight weeks. Ten week old rats, previously fed the untreated purified diet, started to lose weight within two weeks after they were transferred to the same diet treated with ethylene oxide for eighteen hours.

The behavior of the animals (growth failure and early death) suggested that the treated diets might be deficient in thiamine, and assay of the treated purified diets for this vitamin (thiochrome method) indicated extensive thiamine destruction. Animals that were losing weight on such a diet gained an average of 38 g. in body weight within a week after oral thiamine supplementation was begun.

Further studies with thiamine revealed that dispersal in starch afforded protection against the destructive action of ethylene oxide. This protection, however, was overcome when thiamine was dispersed in starch in the presence of choline chloride but not in the presence of choline dihydrogen citrate. The destruction of thiamine in the presence of choline chloride was noted to be associated with an increase in alkalinity.

That thiamine was not the only dietary factor affected by ethylene oxide treatment of the purified diet was evident from the inferior growth that resulted when rats were fed the treated diet fortified with thiamine. Supplementation of the ethylene oxide-treated stock diet with thiamine likewise did not restore normal growth.

Aside from the effect on thiamine, there is at present no explanation for the growth failure of the rats fed ethylene oxide-treated diets. It was noted that both the purified and the natural diet were consumed in lesser quantities after ethylene oxide treatment, which suggests that a reduced palatability can explain, in part at least, the growth failure. The reduction in feed intake, however, was not sufficiently great to account for the lethal effects following feeding of the purified diet.

Sterilization procedures are frequently recognized to influence the nutritive value of foods. Damage so induced must be weighed against the hazards of associated bacterial contamination in nonsterilized foods. The results of Hawk and Mickelsen suggest that before ethylene oxide is considered widely as a food fumigant further studies are needed to establish more clearly the nature of the health failure in animals fed diets fumigated with this agent.

ENZYME CHANGES IN FATTY LIVERS

Since the demonstration over twenty years ago of the profound effects of a deficiency of choline, many workers have investigated the consequences of a deficiency of lipotropic factors in the diet (*Nutrition Reviews* **10,** *187, 208 (1952)*; **11,** *216, 344 (1953)*; **12,** *22, 151, 182 (1954)*). The theory has been advanced that certain morphologic modifications in liver cell particles (mitochondria) may be responsible for osmotic damage and that this may be the cause of the accumulation of fat in the liver cells. An investigation of the content and distribution of cytochrome c in the liver cell has shown that a displacement of this enzyme from mitochondria into the surrounding fluid occurs in fatty livers (M. U. Dianzani and I. Viti, *Biochem. J.* **59,** *141 (1955)*).

A recent study of the distribution of pyridine nucleotides in both normal and fatty liver cells in the rat indicates that changes in the concentration of this factor occur prior to the accumulation of fat in liver cells. Thus a demonstrable biochemical defect is established that further clarifies our understanding of the pathogenesis of fatty livers (M. U. Dianzani, *Biochim. et biophys. acta* **17,** *391 (1955)*).

Dianzani measured the DPN (diphosphopyridine nucleotide) concentration indirectly, by determining the *in vitro* oxidation of glutamate in the presence or in the absence of DPN. DPN is a hydrogen acceptor in the oxidation of glutamate through a specific dehydrogenase with the production of ammonia and alpha-ketoglutarate. With this method it can be demonstrated that in the absence of the hydrogen acceptor (DPN) the oxidation of glutamate is markedly decreased.

In preliminary experiments it was demonstrated that the *in vitro* oxidation of glutamate in both whole liver homogenates and in mitochondria was greatly decreased in fatty livers when DPN was not added to the substrate. While DPN addition to normal liver preparations stimulated glutamate oxidation to some extent (66 per cent for homogenates and 31 per cent for mitochondria), the stimulation in oxidation was much greater in livers of animals that had been injected with carbon tetrachloride, phosphorus, or in those animals that had been fed choline-deficient diets (85 to 120 per cent for homogenates and 84 to 214 per cent for mitochondria). The restoration of oxidative activity of mitochondria by DPN was in general much greater than that for whole liver homogenates. These results suggest that the amount of DPN present in fatty livers is not sufficient for the maximal activity of glutamic dehydrogenase and that this deficiency of DPN is particularly striking in the mitochondria.

Another point of interest was the observed stimulation of glutamate oxidation from added DPN in the mitochondrial fractions of livers from animals that had been treated with fatty liver-inducing agents, or had been fed choline-deficient diets, but had not yet developed fatty livers. For example, after a single phosphorus injection, which did not influence liver lipid content, the oxidation of glutamate was increased 137 per cent by the addition of DPN.

A stimulation of 150 per cent in glutamate oxidation was observed from DPN addition to mitochondrial fractions of livers with normal lipid content from animals that had received a choline-deficient diet for one week.

The distribution of the glutamic dehydrogenase among cell fractions was not influenced by the accumulation of fat in the liver, and normal livers exhibited the following distribution (in per cent): nuclear fraction, 12.2; mitochondria, 63.4; microsomes, 6.4, and supernatant, 18.

A direct measurement of pyridine nucleotides (extinction of the reduced form at 340 millimicrons measured spectrophotometri-

cally) indicated that this substance was greatly reduced in fatty livers. The extent of decrease per gram of tissue was about 36 per cent in the case of rats treated with three injections of carbon tetrachloride, 29 per cent in rats treated with three injections of phosphorus, and 47 per cent in rats fed a choline-deficient diet for four weeks. Correspondingly, nitrogen decreases of 4.3, 4.9 and 8.8 per cent respectively indicated that the decrease in pyridine nucleotides was real. Again, the decrease in pyridine nucleotides was observed to appear before the accumulation of fat in the liver (extent of decrease was 39 and 31 per cent after single injections of carbon tetrachloride and phosphorus, respectively).

From the distribution of pyridine nucleotide among the cell fractions it was evident that with all types of liver-damaging agents used the percentage of nucleotide linked to mitochondria was strongly decreased while that recovered in the supernatant was increased somewhat.

Further studies provided evidence that in the fatty liver the defect appears to be a weak binding between mitochondria and DPN. This evidence was based on the observation that in normal livers mitochondria release only about 30 per cent of their pyridine nucleotide content while those from fatty livers released 70 per cent after one hour incubation at room temperature in 0.25 molar sucrose.

The ratio of oxidized and reduced forms of pyridine nucleotides in normal and fatty livers was also investigated. Whereas the value of this ratio was found to be 3 in normal livers, it was consistently decreased to about 1.6 in fatty livers or in normal livers of animals that had been treated with phosphorus or fed a choline-deficient diet.

The lowered oxidation of glutamate by liver in carbon tetrachloride-poisoned rats and its partial reversal by DPN has also been noted by G. S. Christie and J. D. Judah (*Proc. Roy. Soc. (London) B* **142,** *241 (1955)*).

The decrease in pyridine nucleotides in fatty livers as observed by Dianzani, although less dramatic, has also been noted by H. Frunder in the livers of mice poisoned with carbon tetrachloride (*Z. f. physiol. chem.* **297,** *267 (1954)*).

It is evident from the experiments described above that a marked deficiency of pyridine nucleotides in liver precedes the deposition of fat. The evidence supports the view of a close relationship between the pathogenesis of fatty infiltration and a decrease in this liver cell constituent.

That the oxidation of fatty acids *in vitro* is markedly reduced in liver preparations from choline-deficient rats was established by using C^{14}-labeled stearic or palmitic acid substrates and measuring the production of labeled carbon dioxide (*Nutrition Reviews* **12,** *88 (1954)*).

Whether the decrease in liver pyridine nucleotides is due to an increase in decomposition or a decrease in rate of synthesis remains to be determined.

GLYCEROPHOSPHOLIPID METABOLISM IN LIVER

The structure of lecithin (phosphatidylcholine) has been known for many years, as has the occurrence of enzymes (lecithinases) catalyzing the hydrolytic cleavage of each of the four different types of ester-linkage in the molecule, giving the primary substances from which it is constructed—glycerol, phosphoric acid, choline, and two fatty acid molecules. But aside from a growing conviction that the liver is the principal site of synthesis (*c.f. Nutrition Reviews* **5,** *135 (1947)*; **8,** *147 (1950)*; **9,** *117 (1951)*), little was known five years ago about the steps involved in the construction of this substance

(or the closely related phosphatidylethanolamines and phosphatidylserines) from its components.

It has been shown that at least two syntheses are operative, differing in the order of esterification of phosphoric acid by the glycerol or by the organic base (ethanolamine or choline) moieties. In one, alpha-glyceryl phosphate reacts with fatty acyl CoA (coenzyme A) to yield a phosphatidic acid (A. Kornberg and W. E. Pricer, *J. Biol. Chem.* **204,** *329, 345 (1953)*). The order of addition of the two fatty acid groups is unknown, although the fact that the beta acid is usually saturated while the alpha acid is usually unsaturated would suggest that the two groups are not added indiscriminately. The requirement of CoA and ATP (adenosine triphosphate) in the final juncture of phosphatidic acid with choline (E. P. Kennedy, *J. Am. Chem. Soc.* **75,** *24 (1953)*) has led to the suggestion that phosphatidyl CoA is an intermediate in this pathway.

The other synthesis proceeds via phosphorylcholine, which has been shown to be incorporated intact into the phospholipid molecule (Kornberg and Pricer, *loc. cit.*). E. P. Kennedy and S. B. Weiss (*J. Am. Chem. Soc.* **77,** *250 (1955)*) have demonstrated that cytidine 5′-triphosphate (rather than the more usual adenosine analog, ATP) is involved in this process, via cytidine choline pyrophosphate, which reacts with an unidentified acceptor to yield lecithin. Although alpha, beta-diglycerides (prime suspects for the role of the phosphorylcholine acceptor) do not occur in large concentration in the liver, the fact that such substances are intermediates in the enzymatic hydrolysis of the abundant triglycerides (F. H. Mattson, J. H. Benedict, J. B. Martin, and L. W. Beck, *J. Nutrition* **48,** *335 (1952)*) would indicate their availability.

The demonstration of the presence of free glycerylphosphorylcholine (G. Schmidt *et al.*, *J. Biol. Chem.* **197,** *601 (1952)*) and glycerylphosphorylethanolamine (P. N. Campbell and T. S. Work, *Biochem. J.* **50,** *449 (1952)*) in liver has suggested a third pathway of glycerophospholipid anabolism. However, by studying the relative rates of incorporation of P^{32}-labeled phosphate into these substances and into the related lecithins and phosphatidylethanolamines, R. M. C. Dawson (*Ibid.* **59,** *5 (1955)*) has shown them to be degradation products rather than intermediates. This would appear to be the first indication that such phospholipids are degraded in this way in the liver, although this process is known to be carried out by a number of other enzyme systems. The order of removal of fatty acid moieties remains obscure, but the inimical properties of the lysolecithins would indicate that if such are formed (by initial removal of the alpha-fatty acid), they do not accumulate. Recent advances have thus dispelled a considerable portion of the obscurity which formerly typified our knowledge of the enzymatic synthesis of the physiologically important glycerophospholipids.

THE INTESTINAL ABSORPTION OF AMINO ACIDS

There is a considerable amount of experimental work which has been done mainly on dogs with isolated loops of intestine which indicates that amino acids are absorbed both by diffusion and an active transport mechanism. The evidence for active transport has largely been obtained by measuring the rate of absorption of the natural L-isomer as compared to the D-isomer. In most cases the L-isomer is absorbed at a considerably greater rate. It has also been shown (G. Wiseman, *J. Physiol.* **120,** *63 (1953)*) that the absorption of the L-isomers of alanine, phenylalanine, methionine, histidine and

isoleucine can occur against a concentration gradient. Transport of the D-isomers could not be demonstrated, as was also the case with L-glutamic acid or L-aspartic acid. Similar results were obtained by W. T. Agar, F. J. R. Hird, and G. S. Sidhu *Ibid.* **121,** *255 (1953)*), who also found that the absorption process was inhibited by cyanide and 2,4-dinitrophenol.

Further information on the mechanism of absorption of amino acids has been provided by L. Fridhandler and J. H. Quastel (*Arch. Biochem. Biophys.* **56,** *424 (1955)*). The experimental method which they used was the same as that employed in earlier work on the intestinal absorption of carbohydrates (*Nutrition Reviews* **11,** *280 (1953)*) and consisted of a perfusion apparatus for segments of guinea pig intestine in which the lumen and serosal surface of the intestine are separately perfused. The internal and external perfusion fluids could be sampled at convenient time intervals without stopping the experiment. Sorbose was added to the intraluminal perfusion fluid as an index of simple diffusion which permitted the recognition of leaks in the system.

In this system the rate of appearance of alanine in the serosal solution was three times greater in the case of the L-isomer than in the case of the D-isomer when the concentration of the amino acid perfused through the lumen of the intestinal segment was 0.01 molar.

The effect of concentration on the rate of absorption of alanine was studied using increasing concentrations of DL-alanine in the intraluminal perfusion fluid. At concentrations higher than 0.04 molar a limiting velocity of absorption was reached, from which it was concluded that the transport mechanism for alanine had become saturated. Because earlier *in vivo* observations had suggested that glucose inhibited the absorption of glycine, the effect of glucose on the absorption of alanine was investigated. It was observed that with glucose concentrations less than 0.056 molar, no inhibition of the absorption of alanine occurred. When the luminal glucose concentration was 0.278 molar the transport of alanine fell more than 50 per cent. Because this effect could be reproduced with sorbose it would appear that this action is not specific for glucose but is attributable to the osmotic activity of the solutions.

The absorption of alanine under anaerobic conditions was found to be only one fourth of that under aerobic conditions. The addition of dinitrophenol, 10^{-4} molar, to the luminal fluid lowered the rate of alanine absorption to one-fifth the control level. Phlorizin, 2×10^{-3} molar, however, did not affect the absorption of alanine. A similar concentration will greatly inhibit the absorption of glucose, presumably by inhibiting phosphorylation.

The studies of other workers have suggested that pyridoxine might be concerned in the ability of certain cells to concentrate amino acids. For this reason Fridhandler and Quastel added pyridoxine and desoxypyridoxine to the system, and observed the changes induced in the appearance of alanine in the serosal perfusion medium. Pyridoxine, 10^{-2} molar, had no effect on the rate of alanine absorption, whereas desoxypyridoxine in a similar concentration resulted in a 40 per cent decline in the appearance of alanine in the serosal medium. The authors were, however, unsuccessful in reversing the inhibition produced by desoxypyridoxine by addition of pyridoxine, 10^{-2} molar, pyridoxamine phosphate, 3×10^{-3} molar, or pyridoxal phosphate, 1.2×10^{-2} molar. Because desoxypyridoxine also inhibited the absorption of glucose and the conversion of fructose to glucose by the intestinal mucosa, it cannot be concluded that the effect of this vitamin antagonist is limited to amino acid absorption.

Fridhandler and Quastel, using their system, also obtained confirmation of the active absorption of L-histidine and L-phenyl-

alanine and the low rate of absorption of L-glutamic acid. Glutamine was found to be absorbed at a rapid rate and only glutamine appeared in the serosal medium. The perfusion of the mucosal surface with glutamine and pyruvate did not lead to the appearance of alanine in the serosal medium by the process of transamination.

Aspartic acid, like glutamic acid, was found to be absorbed slowly by the isolated guinea pig intestine. Asparagine was absorbed "actively," giving rise to aspartic acid plus ammonia in the serosal medium, but no asparagine.

In conclusion, it is apparent that some but not all amino acids are absorbed by an active transport mechanism that is specific for the L-type isomer. The transport mechanism does not apply to the dicarboxylic amino acids glutamic or aspartic acid unless the second carboxylic acid is blocked by an amide group. It is possible that pyridoxine may be involved in the transport system, although this has not been established.

LONG-TERM REGULATION OF FOOD INTAKE

Available information suggests many variables are involved in the regulation of food intake. These include environmental temperature (J. R. Brobeck, *Yale J. Biol. Med.* **20,** *545 (1948)*; J. Mayer, *Ann. N. Y. Acad. Sci.* **63,** *15 (1955)*), the integrity of several hypothalamic structures (*Nutrition Reviews* **2,** *105 (1944)*; **11,** *55 (1953)*), palatal stimuli and gastrointestinal distension (H. D. Janowitz and M. I. Grossman, *Am. J. Physiol.* **159,** *143 (1949)*), emotional and conditioned responses (H. Bruch, *Ann. N. Y. Acad. Sci.* **63,** *68 (1955)*) and circulating metabolites (Mayer and M. W. Bates, *Am. J. Physiol.* **168,** *812 (1952)*; *Nutrition Reviews* **10,** *280 (1952)*), as well as genetically determined metabolic pathways.

In many of the studies heretofore published, an underlying assumption seems to have been that changes in these variables are promptly reflected in appetite. However, a recent study of H. D. Janowitz and F. Hollander (*Ann. N. Y. Acad. Sci.* **63,** *56 (1955)*) places emphasis on the rate, as well as the precision of long term regulation, of caloric intake under conditions of varied caloric need.

An earlier study of this type was carried out by I. Share, E. Martyniuk, and M. I. Grossman (*Am. J. Physiol.* **169,** *229 (1952)*). Dogs with fistulas were fed 33, 50, 66 and 133 per cent of their previous voluntary daily caloric intake intragastrically, four hours after each daily ad libitum feeding, for a period of three to four weeks. Voluntary eating seemed to be lowered in proportion to the intragastric feeding, but even an intragastric level of above 100 per cent (133 per cent for one week) did not completely abolish oral intake.

The present report by Janowitz and Hollander describes the effect of 50, 100 and 175 per cent intragastric feeding carried out for a period of three months in female mongrel dogs with fistulas. The dogs were allowed to eat ad libitum once each day, in the following manner: Excessive amounts of a commercial whole dog food were offered in weighed pans and left for forty-five minutes. The caloric value was derived as follows: 38.3 per cent from protein, 23.4 per cent from fat, and 38.3 per cent from carbohydrate.

After a control period during which the average daily food intake and the weekly body weight were stabilized, the various percentages of the mean voluntary caloric intake were given intragastrically six hours before each oral feeding. The caloric density of the intragastric material was adjusted by the addition of water so that the volume equaled the daily volume of food eaten voluntarily by each animal during the adjustment period.

The effect of intragastric feeding on oral intake was similar in all dogs. Following a transitional adjustment period (average of 4.4 weeks) during which oral intake decreased progressively eating behavior reached a new plateau. Eating was readjusted with considerable precision to attain complete compensation of caloric supply. During the 50 per cent level experiments the mean daily intake slightly exceeded the mean daily deficit in calories given by fistula. During the 100 per cent level experiments the dogs still ate a small amount, but with intragastric feeding at the 175 per cent level, oral feeding was virtually abolished. The small amount of food intake which did take place was to be expected on the basis of conditioned reflex and normal curiosity.

With cessation of intragastric feeding, eating increased in compensation and, after a period of adjustment (average of 3.6 weeks), the amount of food ingested closely approximated that eaten during the initial control period.

During the period of intragastric feeding, body weight was maintained at statistically constant levels in the 50 per cent level experiments, in one dog of 3 in the 100 per cent experiments, and in 2 dogs of 3 in the 175 per cent level experiments. This constancy in weight at the 175 per cent level was not expected and is not explained. Share *et al.* (*loc. cit.*) also observed this phenomenon in dogs force-fed at the 133 per cent level.

Although time is required for their action, there can be little doubt that regulating mechanisms are capable of making fine adjustments of caloric intake. The experiments of Janowitz and Hollander do not indicate just what mechanisms may be involved, although palatal stimuli, gastric distension and temperature were eliminated as salient factors in the long-term adjustments of eating behavior. The authors suggest that a systemic factor was influencing the integrative activity of the central nervous system, especially that of the hypothalamus, which has been shown by J. L. Strominger and J. R. Brobeck (*Yale J. Biol. Med.* **25,** *383* (*1953*)) and others to be involved in physiologic regulations of food intake.

Total calories (Strominger and Brobeck, *loc. cit.*), blood glucose, and blood and depot lipid (Mayer, *loc. cit.*) have been suggested as "intrinsic food factors" capable of influencing the hypothalamus directly and indirectly and thus regulating the food intake. However, the studies of Strominger and Brobeck have resulted in rejection of the "calorie *per se*" theory. Also, although blood glucose seems to play a definite role in regulation of appetite, it is not yet clear whether lowered blood glucose acts on other mechanisms than the hypothalamus. Studies carried out by Mayer and Bates (*loc. cit.*) indicated that although lesions of certain areas of the hypothalamus in dogs seemed to be responsible for extreme voraciousness immediately after the operations, lowered blood glucose prevailed as a long-term effect of the lesions and may have served as the stimulus for other regulating mechanisms.

NOTES

Letter to the Editor

Dear Sir:

In a recent Note which appeared in *Nutrition Reviews* (**13,** *287* (*1955*)) the problem of inactivation of trypsin inhibitor by duodenal juice was discussed. M. L. Haanes and P. György (*Am. J. Physiol.* **166,** *441* (*1951*)) reported that the pancreatic anti-fatty liver fraction studied by them showed proteolytic activity after treatment with duodenal juice. They interpreted this as possibly being due to inactivation of trypsin inhibitor in the pancreatic extract by enterokinase in the duodenal juice. However, P. H. Mars, R. J. Peanasky, and M. Laskowski (*Proc. Soc. Exp. Biol. Med.* **82,** *384* (*1953*)) showed that purified enterokinase was unable to overcome the action of crystalline pancreatic trypsin inhibitor. They therefore proposed that the results of Haanes and György be explained by assuming that the pancreatic anti-fatty liver extract contained trypsinogen which was activated by the enterokinase of the duodenal juice. This explanation was supported when Peanasky and Laskowski (*Ibid.* **83,** *686* (*1953*)) showed that the pancreatic extract studied by Haanes and György contained trypsinogen.

Recently M. H. Kalser and M. I. Grossman (*Gastroenterology* **29,** *35* (*1955*)) demonstrated that extracts of duodenal mucosa were capable of counteracting the action of pancreatic trypsin inhibitor.

It would appear that all of the observations to date can be reconciled if it is assumed that duodenal juice or duodenal extracts are capable of inactivating pancreatic trypsin inhibitor but that enterokinase is not the constituent responsible for this effect.

MORTON I. GROSSMAN
Chief, Section on Gastroenterology
Veterans Administration Center
Los Angeles 25, California

Glucose-6-Phosphatase

Using the method developed by M. A. Swanson (*J. Biol. Chem.* **184,** *647* (*1950*)), R. C. Harris and C. Olmo (*J. Clin. Invest.* **33,** *1204* (*1954*)) have studied the glucose-6-phosphatase activity of liver and kidney tissue obtained, in the case of liver, by surgical biopsy or at autopsy, and in the case of kidney and also heart, at autopsy from a patient who had glycogen storage disease.

Control determinations were made on the livers of 9 patients with disease presumably not affecting this organ. Three of these patients (who had pyloric stenosis) were operated upon and the tissue was removed at that time. The remaining 6 patients died of various conditions at ages ranging from 40 hours to 34 days. It was found best to express the glucose-6-phosphatase activity as milligrams of phosphorus per gram of nitrogen rather than per gram of wet tissue or other standard. In this way these normal livers varied from 165 to 299 mg. of phosphorus per gram of nitrogen, averaging 214 mg. of phosphorus with a standard deviation of ± 45.

The glucose-6-phosphatase activity was then determined on the livers of a group of 22 patients. In 9 the tissue was obtained by biopsy and in 14 at autopsy (including one in whom the liver was studied both at biopsy and subsequently at autopsy). In these patients various types of liver disease were observed, such as cirrhosis, pericholangitis (tissue inflammation around bile ducts), hepatitis, and passive congestion. In general, the values for glucose-6-phosphatase activity were well below those in which the liver was presumed normal. The lowest values were 6 mg. of phosphorus per milligram of nitrogen for a 3 year old child with Laennec's cirrhosis, and 26 mg. of phosphorus per milligram of nitrogen in 2 children, one with neuroblastoma of the liver and the other with reticuloendotheliosis.

The enzyme activity was studied in the livers of 2 patients with glycogen storage disease of the liver. In both, the enzyme activity was greatly reduced, being close to that of the patient with cirrhosis noted above. These patients with glycogen storage disease of the liver had a markedly reduced enzyme activity of the kidney cortex.

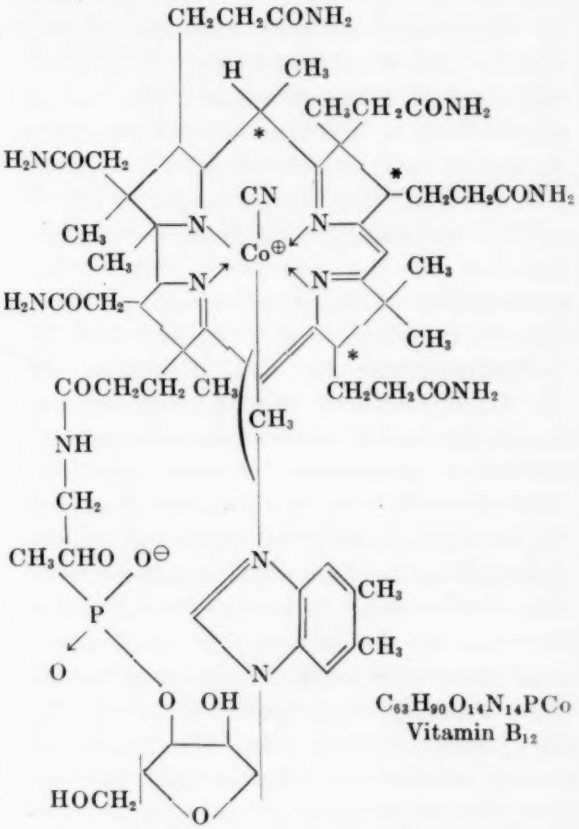
Finally, a 2 month old infant was studied who, although suspected of having typical glycogen storage disease, turned out to have large deposits of glycogen in heart and skeletal muscle. In this instance the liver glucose-6-phosphatase activity was almost 3 standard deviations above normal.

It thus appears that in glycogen storage disease of the liver, glucose-6-phosphatase activity is almost absent, and that it is usually reduced in diseases affecting the liver. These results correlate well with the known activity of this enzyme in converting glucose-6-phosphate derived from glycogen to glucose.

The Structure of Vitamin B_{12}

The isolation of crystalline vitamin B_{12} was announced eight years ago by American workers at the Merck laboratories and by Lester Smith in England (*see Nutrition Reviews* **6,** *245, 291 (1948)*). It was soon observed that the crystalline vitamin contained cobalt and was a large molecule. Since the original isolation, work has been progressing on the structure of the vitamin both in this country and in England. The complete structure of vitamin B_{12} has now been determined (D. C. Hodgkin and coworkers, *Nature* **176,** *325 (1955)*; R. Bonnett *et al., Ibid.* **176,** *328 (1955)*).

These workers conducted extensive x-ray analyses on the hexacarboxylic acid derived from vitamin B_{12}. The results of these x-ray studies were analyzed by the National Bureau of Standards western automatic computator in Los Angeles. These data, taken in conjunction with the degradation studies of the Merck group (F. A. Kuehl, C. H. Shunk, and K. Folkers, *J. Am. Chem. Soc.* **77,** *251 (1955)*), permitted complete formulation of the B_{12} molecule. The proposed structure is as follows:



$C_{63}H_{90}O_{14}N_{14}PCo$
Vitamin B_{12}

This work is important not only because it adds to our fundamental knowledge, but it also may pave the way for the production of metabolic antagonists of the vitamin B_{12} molecule. Such analogues would undoubtedly aid in the elucidation of the biochemical role of vitamin B_{12}.

The size and complexity of the B_{12} molecule indicates that its chemical synthesis will be difficult, and probably not commercially practical because of the ease of production by microorganisms.

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